Clinical Validation of a Novel High-Sensitivity Cardiac Troponin I Assay for Early Diagnosis of Acute Myocardial Infarction.

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Abstract: BACKGROUND Clinical performance of the novel high-sensitivity cardiac troponin I (Siemens-hs-cTnI-Centaur) assay is unknown. We aimed to clinically validate the Siemens-hs-cTnI-Centaur assay and develop 0/1-h and 0/2-h algorithms. METHODS We enrolled patients presenting to the emergency department with symptoms suggestive of acute myocardial infarction (AMI). Final diagnoses were centrally adjudicated by 2 independent cardiologists including all clinical information twice: first, using serial hs-cTnT (Roche-Elecsys, primary analysis); second, using hs-cTnI (Abbott-Architect, secondary analysis) measurements in addition to the clinically applied (hs)-cTn. Siemens-hs-cTnI-Centaur was measured at presentation, 1 h, and 2 h. The primary objective was a direct comparison of diagnostic accuracy, quantified by the area under the ROC curve (AUC), of Siemens-hs-cTnI-Centaur vs the 2 established hs-cTn assays (Roche-hs-cTnT-Elecsys, Abbott-hs-cTnI-Architect). Secondary objectives included the development of Siemens-hs-cTnI-Centaur-specific 0/1-h and 0/2-h algorithms. RESULTS AMI was the final diagnosis in 318 of 1755 (18%) patients (using Roche-hs-cTnT-Elecsys for adjudication). The AUC at presentation for Siemens-hs-cTnI-Centaur was 0.94 (95% CI, 0.92-0.96) and comparable with 0.95 (95% CI, 0.93-0.97) for Roche-hs-cTnT-Elecsys and 0.93 (95% CI, 0.90-0.96) for Abbott-hs-cTnI-Architect. Applying the derived Siemens-hs-cTnI-Centaur 0/1-h algorithm to the validation cohort, 46% of patients were ruled out (sensitivity, 99.1%; 95% CI, 95.3-100), and 18% of patients were ruled in (specificity, 94.1%; 95% CI, 91.8-95.9). The Siemens-hs-cTnI-Centaur 0/2-h algorithm ruled out 55% of patients (sensitivity, 100%; 95% CI, 94.1-100), and ruled in 18% of patients (specificity, 96.0%; 95% CI, 93.1-97.9). Findings were confirmed in the secondary analyses using serial measurements of Abbott-hs-cTnI-Architect for adjudication. CONCLUSIONS Diagnostic accuracy and clinical utility of the novel Siemens-hs-cTnI-Centaur assay are high and comparable with the established hs-cTn assays. ClinicalTrials.gov Identifier: NCT00470587.

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Clinical Validation of a Novel High-Sensitivity Cardiac Troponin I Assay for Early Diagnosis of Acute Myocardial Infarction

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BACKGROUND: Clinical performance of the novel high-sensitivity cardiac troponin I (Siemens-hs-cTnI-Centaur) assay is unknown. We aimed to clinically validate the Siemens-hs-cTnI-Centaur assay and develop 0/1-h and 0/2-h algorithms.

METHODS: We enrolled patients presenting to the emergency department with symptoms suggestive of acute myocardial infarction (AMI). Final diagnoses were centrally adjudicated by 2 independent cardiologists including all clinical information twice: first, using serial hs-cTnT (Roche-Elecsys, primary analysis); second, using hs-cTnI (Abbott-Architect, secondary analysis) measurements in addition to the clinically applied (hs)-cTn. Siemens-hs-cTnI-Centaur was measured at presentation, 1 h, and 2 h. The primary objective was a direct comparison of diagnostic accuracy, quantified by the area under the ROC curve (AUC), of Siemens-hs-cTnI-Centaur vs the 2 established hs-cTn assays (Roche-hs-cTnT-Elecsys, Abbott-hs-cTnI-Architect). Secondary objectives included the development of Siemens-hs-cTnI-Centaur-specific 0/1-h and 0/2-h algorithms.

RESULTS: AMI was the final diagnosis in 318 of 1755 (18%) patients (using Roche-hs-cTnT-Elecsys for adjudication). The AUC at presentation for Siemens-hs-cTnI-Centaur was 0.94 (95% CI, 0.92–0.96) and comparable with 0.95 (95% CI, 0.93–0.97) for Roche-hs-cTnT-Elecsys and 0.93 (95% CI, 0.90–0.96) for Abbott-hs-cTnI-Architect. Applying the derived Siemens-hs-cTnI-Centaur 0/1-h algorithm to the validation cohort, 46% of patients were ruled out (sensitivity, 99.1%; 95% CI, 95.3–100), and 18% of patients were ruled in (specificity, 94.1%; 95% CI, 91.8–95.9). The Siemens-hs-cTnI-Centaur 0/2-h algorithm ruled out 55% of patients (sensitivity, 100%; 95% CI, 94.1–100), and ruled in 18% of patients (specificity, 96.0%; 95% CI, 93.1–97.9). Findings were confirmed in the secondary analyses using serial measurements of Abbott-hs-cTnI-Architect for adjudication.

CONCLUSIONS: Diagnostic accuracy and clinical utility of the novel Siemens-hs-cTnI-Centaur assay are high and comparable with the established hs-cTn assays.

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Up to 10% of all emergency department (ED)[11] consultations are for patients with symptoms suggestive of acute myocardial infarction (AMI) *(1)*. Rapid identification of AMI as a life-threatening disorder is important for the early initiation of appropriate evidence-based therapy *(2)*. Electrocardiography (ECG) and cardiac troponin (cTn) form the diagnostic cornerstones and complement

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Disclaimer: The authors designed the study, gathered and analyzed the data, vouched for the data and analysis, wrote the paper, and decided to publish. Drs. Boeddinghaus, Twerenbold, Nestelberger, Badertscher, Rubini Gimenez, Wildi, Puelacher, Reichlin, and Mueller had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

[11] Nonstandard abbreviations: ED, emergency department; AMI, acute myocardial infarction; ECG, electrocardiography; cTn, cardiac troponin; hs-cTn, high-sensitivity cardiac troponin; LoD, limit of detection; ESC, European Society of Cardiology; NPV, negative predictive value; CART, classification and regression tree; PPV, positive predictive value; MACE, major adverse cardiac event; AUC, area under the ROC curve; IQR, interquartile range; STEMI, ST-segment elevation myocardial infarction.

clinical assessment in the early rule-out or rule-in of AMI *(2–4)*.

The introduction of high-sensitivity cardiac troponin (hs-cTn) assays enabled reliable measurement of cTn concentrations in the reference interval *(5)* and increased diagnostic accuracy for AMI at presentation *(6)*. Two hs-cTn assays, Roche-hs-cTnT-Elecsys and Abbott-hs-cTnI-Architect, have been extensively investigated in large diagnostic studies, including the successful derivation and validation of early 0/1-h and 0/2-h triage algorithms *(3, 7–20)*.

More recently, the novel hs-cTnI-Centaur assay was developed. It constitutes only the third hs-cTn assay to become available for clinical use. Before its possible implementation into routine clinical care, its performance in patients presenting with suspected AMI must be thoroughly examined. Therefore, we set out to compare its diagnostic accuracy with that of the 2 established hs-cTn assays, and derived and validated assay-specific 0/1-h and 0/2-h algorithms.

Materials and Methods

STUDY DESIGN AND POPULATION

*A*dvantageous *P*redictors of *A*cute *C*oronary Syndrome *E*valuation (APACE) is an ongoing prospective international multicenter study with 12 centers in 5 countries aiming to advance the early diagnosis of AMI (ClinicalTrials.gov registry, number NCT00470587) *(6, 7, 9, 11, 12, 14, 15, 21–23)* (see the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol64/issue9).

ADJUDICATION OF THE FINAL DIAGNOSIS

Central adjudication of the final diagnosis was performed by 2 independent cardiologists applying the universal definition of AMI using 2 sets of data: first, all available medical records obtained during clinical care including cardiac imaging; second, study-specific assessments including serial hs-cTnT concentrations. To address the uncommon, but previously described, phenomenon of discrepant results for hs-cTnT and hs-cTnI, we performed a second adjudication using serial hs-cTnI (rather than hs-cTnT) blood concentrations from study samples (see the online Data Supplement).

INVESTIGATIONAL hs-cTn MEASUREMENTS

For determination of Siemens-hs-cTnI-Centaur, we used blood samples collected into serum containers, and for determination of Abbott-hs-cTnI-Architect and Roche-hs-cTnT-Elecsys, we used blood samples collected into serum containers or lithium heparin plasma containers, respectively. Study-specific blood draws were performed immediately after informed consent had been obtained at ED presentation and additionally at 1, 2, 3, and 6 h. Serial sampling was discontinued when a patient was released or transferred to the catheter laboratory for acute treatment. After centrifugation, samples were frozen at −80 °C until assayed in a blinded fashion at a dedicated core laboratory.

According to the manufacturer, the hs-cTnI-Centaur assay (ADVIA Centaur TNIH, Siemens Healthcare) has a population 99th percentile concentration (both sexes) of 47 ng/L with a corresponding CV of <5%. The 99th percentiles for men and women are 58 ng/L and 39 ng/L, respectively. Limit of blank, limit of detection (LoD), and limit of quantification have been determined to be 0.9 ng/L, 2.2 ng/L, and 2.5 ng/L, respectively. The assay is a dual-capture sandwich immunoassay using magnetic latex particles and a proprietary acridinium ester for chemiluminescence detection. The detection reagent is a recombinant sheep Fab antibody covalently linked to a trisulfo propyl acridinium ester–BSA conjugate. Trisulfo propyl acridinium ester is a new generation of high-yield acridinium esters developed for enhanced chemiluminescent detection. Simultaneous addition of solid-phase reagent and detection reagent to the sample forms a classic sandwich immune complex, which is subsequently washed. Chemiluminescence is initiated and measured. Relative light units are directly proportional to the cTnI concentration. The time to first result is 18 min. The assay meets the current IFCC recommendations for hs-cTn assays *(24, 25)*.

The hs-cTnT-Elecsys assay (Elecsys 2010, Roche Diagnostics) has a 99th percentile concentration of 14 ng/L with a corresponding CV of 10% at 13 ng/L *(5)*. Limit of blank and LoD have been determined to be 3 ng/L and 5 ng/L, respectively *(5)*. The hs-cTnI-Architect assay (ARCHITECT STAT high-sensitivity troponin I, Abbott Laboratories) has a 99th percentile concentration of 26.2 ng/L with a corresponding CV of <5% and an LoD of 1.9 ng/L *(26–28)*. Estimated glomerular filtration rate was calculated using the abbreviated Modification of Diet in Renal Disease formula *(29)*.

DERIVATION OF THE hs-cTnI-CENTAUR 0/1h-ALGORITHM

The hs-cTnI-Centaur 0/1-h algorithm was developed in a derivation sample of randomly selected (1:1 fashion) patients with available hs-cTnI-Centaur measurements at baseline and after 1 h according to the central adjudication by 2 independent cardiologists using all clinical information including cardiac imaging and serial hs-cTnT-Elecsys concentrations (primary adjudication). The 0/1-h algorithm incorporates hs-cTnI-Centaur concentrations at presentation and absolute hs-cTnI-Centaur changes within 1 h ($\text{hs-cTnI-Centaur}_{1h} - \text{hs-cTnI-Centaur}_{0h}$), as well as time since chest pain onset to reflect the concept of the current hs-cTn 0/1-h algorithms suggested by the European Society of Cardiology (ESC) *(3)* (see Fig. 4 in the online Data Supplement). Selection of these parameters was based on the high diagnostic accuracy of the

combination of blood concentrations at presentation with absolute changes for rule-out and rule-in of AMI *(7, 8, 11, 12, 14, 15, 21, 30, 31)*. Optimal thresholds for rule-out were selected to allow for a minimal sensitivity and negative predictive value (NPV) of 99% and were independent from the assay package insert-specified thresholds. Optimal thresholds for rule-in were obtained based on a classification and regression tree (CART) analysis targeting a minimal positive predictive value (PPV) of 70% *(32, 33)*. Nodes in the CART tree were constrained to have a minimal number of 20 cases in parent and child nodes. If a predefined target performance was missed in the derivation sample using the CART-derived thresholds, thresholds were changed stepwise until the predefined performance was fulfilled.

DERIVATION OF THE hs-cTnI-CENTAUR 0/2h-ALGORITHM

The hs-cTnI-Centaur 0/2-h algorithm was developed in a derivation sample randomly selected (2:1 fashion to compensate for the slightly lower number of patients with available 2-h vs 1-h samples and to ensure a sufficient number of patients in the derivation cohort) of patients with available hs-cTnI-Centaur measurements at ED presentation and after 2 h (see the online Data Supplement) according to the central adjudication by 2 independent cardiologists using all clinical information including cardiac imaging and serial hs-cTnT-Elecsys concentrations (primary adjudication).

VALIDATION OF THE hs-cTnI-CENTAUR 0/1-h AND 0/2-h ALGORITHM

The algorithms developed in the derivation samples were tested for their diagnostic accuracy in internal validation samples consisting of the remaining subjects. The optimal decision values derived in the derivation sample were rounded to give whole values in nanograms per liter. The first validation was done according to the central adjudication by 2 independent cardiologists using all clinical information including cardiac imaging and serial hs-cTnT-Elecsys concentrations. The secondary validation was done according to the central adjudication by 2 independent cardiologists using all clinical information including cardiac imaging and serial hs-cTnI-Architect concentrations.

FOLLOW-UP AND CLINICAL END POINTS

Clinical follow-up is described in detail in the online Data Supplement. The coprimary prognostic end points were overall survival after 30 days and 2 years. The secondary prognostic end point was major adverse cardiac events (MACE) defined as the composite of all-cause mortality, AMI, cardiogenic shock, ventricular tachyarrhythmias, or higher degree atrioventricular block at 30 days.

STATISTICAL ANALYSIS

For the primary analysis, serial hs-cTnT-Elecsys concentrations were used for final adjudication. For the secondary analysis, serial hs-cTnI-Architect concentrations were used for final adjudication. ROC curves were constructed to assess the sensitivity and specificity throughout the hs-cTn concentrations to compare the ability to diagnose AMI. Subgroup analyses were performed in patients presenting to the ED very soon ($\leq$2 h), soon ($\leq$3 h), and late ($>$3 h) after chest pain onset/maximum, as well as in women and men. We further included analysis using sex-specific cutoffs and investigated the performance of the ESC 0/3-h algorithm. Biological equivalent concentrations were determined by plotting log-transformed hs-cTnI-Centaur and hs-cTnI-Architect or hs-cTnT-Elecsys concentrations from the same sample *(22)*. The areas under the ROC curves (AUC) were compared as recommended by DeLong et al. *(34)* or by *z*-statistic, as appropriate (see the online Data Supplement).

Safety was assessed as the NPV and the sensitivity of AMI for rule-out; accuracy for rule-in was assessed as the PPV and specificity of AMI; and efficacy was quantified as the percentage of patients triaged toward rule-out or rule-in for AMI within 1 h or 2 h.

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (SPSS) and MedCalc 17.6 (MedCalc Software).

Results

CHARACTERISTICS OF PATIENTS

From April 2006 to February 2013, 1755 patients eligible for this analysis were enrolled (see Fig. 1 in the online Data Supplement). Thirty-four percent of patients presented to the ED within the first 3 h after chest pain onset. Baseline characteristics of all patients are shown in Table 1, and those of patients in the derivation and validation cohorts are shown in Table 2 of the online Data Supplement.

ADJUDICATED FINAL DIAGNOSIS

Of 1755 patients, the adjudicated final diagnosis was AMI in 318 (18%), unstable angina in 156 (9%), cardiac symptoms of origin other than coronary artery disease (e.g., tachyarrhythmia, takotsubo cardiomyopathy, heart failure, and myocarditis) in 238 (14%), noncardiac symptoms in 968 (55%), and unknown in 75 (4%). Final diagnoses according to the second final adjudication including hs-cTnI (Architect) were similar (see the online Data Supplement).

CONCENTRATIONS OF hs-cTnI-CENTAUR AT PRESENTATION ACCORDING TO FINAL DIAGNOSES

Concentrations of hs-cTnI-Centaur at ED presentation were significantly higher in patients with AMI as compared with patients with other final diagnoses ($P <$ 0.001). Median concentrations of hs-cTnI-Centaur in

Table 1. Baseline characteristics of the patients.[a]

	All patients (n = 1755)	AMI (n = 318)	No AMI (n = 1437)	*P* value
Age, years	62 (49–75)	72 (59–80)	60 (47–73)	<0.001
Male sex, n (%)	1216 (69)	231 (73)	985 (69)	0.15
Time from CPO[b] to first blood draw, h	5 (3–10)	5 (3–11)	5 (3–10)	0.41
Early presenters (within 3 h after CPO)	603 (34%)	106 (33%)	497 (35%)	0.67
Risk factors, n (%)				
Hypertension	1087 (62)	255 (80)	832 (58)	<0.001
Hypercholesterolemia	876 (50)	219 (69)	657 (46)	<0.001
Diabetes	317 (18)	84 (27)	233 (16)	<0.001
Current smoking	442 (25)	77 (24)	365 (25)	0.69
History of smoking	658 (38)	131 (42)	527 (37)	0.11
History, n (%)				
Coronary artery disease	619 (35)	160 (50)	459 (32)	<0.001
Previous MI	409 (23)	107 (34)	302 (21)	<0.001
Previous revascularization	479 (27)	116 (37)	363 (25)	<0.001
Peripheral artery disease	116 (7)	48 (15)	68 (5)	<0.001
Previous stroke	89 (5)	24 (8)	65 (5)	0.03
ECG findings, n (%)				
Left bundle branch block	55 (3)	15 (5)	40 (3)	0.07
ST-segment depression	156 (9)	88 (28)	68 (5)	<0.001
T-wave inversion	152 (9)	39 (12)	113 (8)	0.01
No significant ECG abnormalities	1358 (77)	169 (53)	1189 (83)	<0.001
Body mass index, kg/m^2	27 (24–30)	26 (24–29)	27 (24–30)	0.47
Laboratory findings				
Creatinine clearance, mL/min/m^2	84 (68–100)	72 (54–89)	86 (70–102)	<0.001
Chronic medication, n (%)				
Aspirin	638 (36)	155 (49)	483 (34)	<0.001
Vitamin K antagonists	143 (8)	31 (10)	112 (8)	0.25
β-blockers	595 (34)	137 (43)	458 (32)	<0.001
Statins	613 (35)	140 (44)	473 (33)	<0.001
ACEIs/ARBs[c]	656 (37)	167 (53)	489 (34)	<0.001
Calcium antagonists	236 (13)	59 (19)	177 (12)	0.003
Nitrates	200 (11)	65 (20)	135 (9)	<0.001

[a] Numbers are presented as median (IQR) or numbers (%).
[b] Chest pain onset.
[c] Angiotensin-converting-enzyme inhibitors/angiotensin receptor blockers.

patients with AMI were 235 ng/L [interquartile range (IQR), 39–1018]; with unstable angina, 8.5 ng/L (IQR, 5.0–17); with cardiac but not coronary disease, 12 ng/L (IQR, 4.7–36); with noncardiac disease, 4 ng/L (IQR, 2.4–7.6); and with unknown diagnosis, 4.2 ng/L (IQR, 2.8–7.0; Fig. 1). Similar findings emerged according to the second final adjudicated diagnosis including hs-cTnI (Architect; see Fig. 2 in the online Data Supplement).

DIAGNOSTIC ACCURACY FOR ACUTE MYOCARDIAL INFARCTION

The diagnostic accuracy of measurements obtained at presentation, as quantified by AUCs, for the hs-cTnI-Centaur assay was 0.94 (95% CI, 0.92–0.96) and comparable with hs-cTnT-Elecsys at 0.95 (95% CI, 0.93–0.97) and hs-cTnI-Architect at 0.93 (95% CI, 0.90–0.96), respectively ($P = 0.370$ and $P = 0.780$ for direct comparisons; Fig. 2A). For hs-cTnI-Centaur, the AUCs for

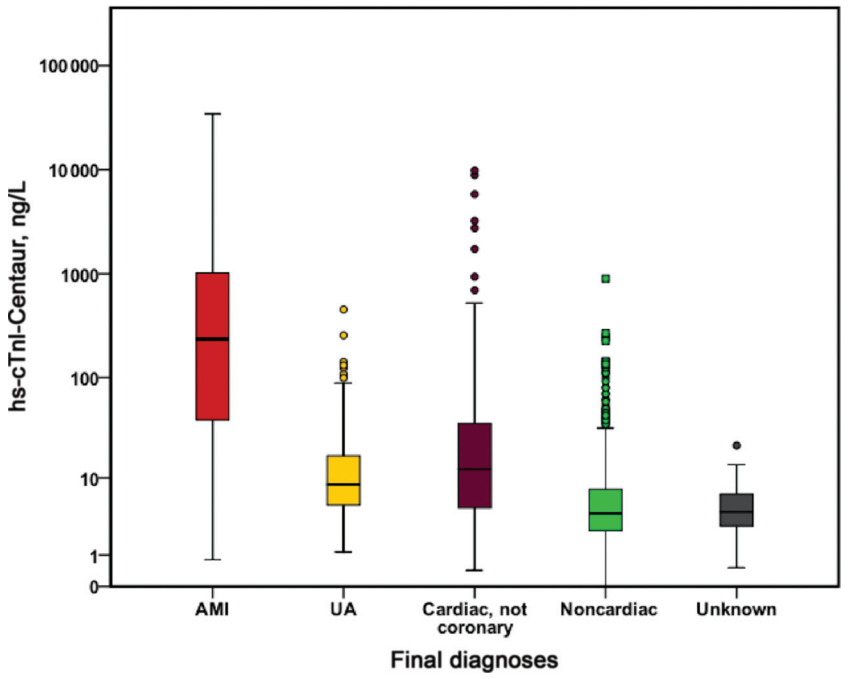

Fig. 1. Boxplots showing concentrations of hs-cTnI-Centaur at presentation according to the final diagnosis.
Boxes represent medians and IQRs, while whiskers display the smallest and the largest nonoutliers. Rings display outliers further than 1.5 IQRs, and boxes display outliers further than 3 IQRs from the respective end of the box. UA, unstable angina.

concentrations at 1 h, 2 h, and 3 h were 0.95 (95% CI, 0.93–0.96), 0.95 (95% CI, 0.94–0.97), and 0.97 (95% CI, 0.95–0.99), respectively (see Table 3A in the online Data Supplement). Similar findings emerged according to the second final adjudicated diagnosis including hs-cTnI (Architect; see Fig. 3A in the online Data Supplement). The diagnostic performances of uniform and sex-specific cutoffs are summarized in Table 2, and detailed information is given in the Results section of the online Data Supplement.

SUBGROUP ANALYSES ACCORDING TO TIME SINCE CHEST PAIN ONSET AND SEX

Diagnostic accuracy at presentation was also similar in the predefined subgroups (Fig. 2B; see also Table 3B and the Results section of the online Data Supplement). Again, similar findings emerged according to the second final adjudicated diagnosis including hs-cTnI (Architect; see Fig. 3B of the online Data Supplement).

hs-cTnI-CENTAUR 0/1-h ALGORITHM

The diagnostic performance of the hs-cTnI-Centaur 0/1-h algorithm in the derivation cohort is shown in Fig. 3A here and Fig. 5A of the online Data Supplement.

VALIDATION OF THE hs-cTnI-CENTAUR 0/1-h ALGORITHM

Applying the derived optimal cutoff levels to the internal validation cohort, 313 of 675 patients (46%) could be classified as rule-out with a corresponding NPV of 99.7% (95% CI, 97.8–100) and a sensitivity of 99.1% (95% CI, 95.3–100; see Fig. 3B here and Fig. 5B of the online Data Supplement). Direct rule-out based on a single hs-cTnI-Centaur concentration at presentation was feasible in 111 of 675 patients (16%). One patient with AMI was missed out of 675 patients with suspected AMI in the validation sample (see Table 4 in the online Data Supplement for detailed patient characteristics). The 0/1-h algorithm classified 120 of 675 patients (18%) as rule-in with a corresponding PPV of 72.5% (95% CI, 63.6–80.3) and a specificity of 94.1% (95% CI, 91.8–95.9). Direct rule-in based on a single hs-cTnI-Centaur concentration at presentation was feasible in 79 of 675 patients (12%). Overall, the hs-cTnI-Centaur 0/1-h algorithm allowed a definite diagnosis after 1 h in 433 of 675 patients (64%; either rule-out or rule-in). The remaining 242 of 675 patients (36%) were classified to observe with an AMI prevalence of 11% (95% CI, 8–15). Similar findings emerged when assessing the diagnostic performance of the hs-cTnI-Centaur 0/1-h algorithm in the validation cohort using the second final

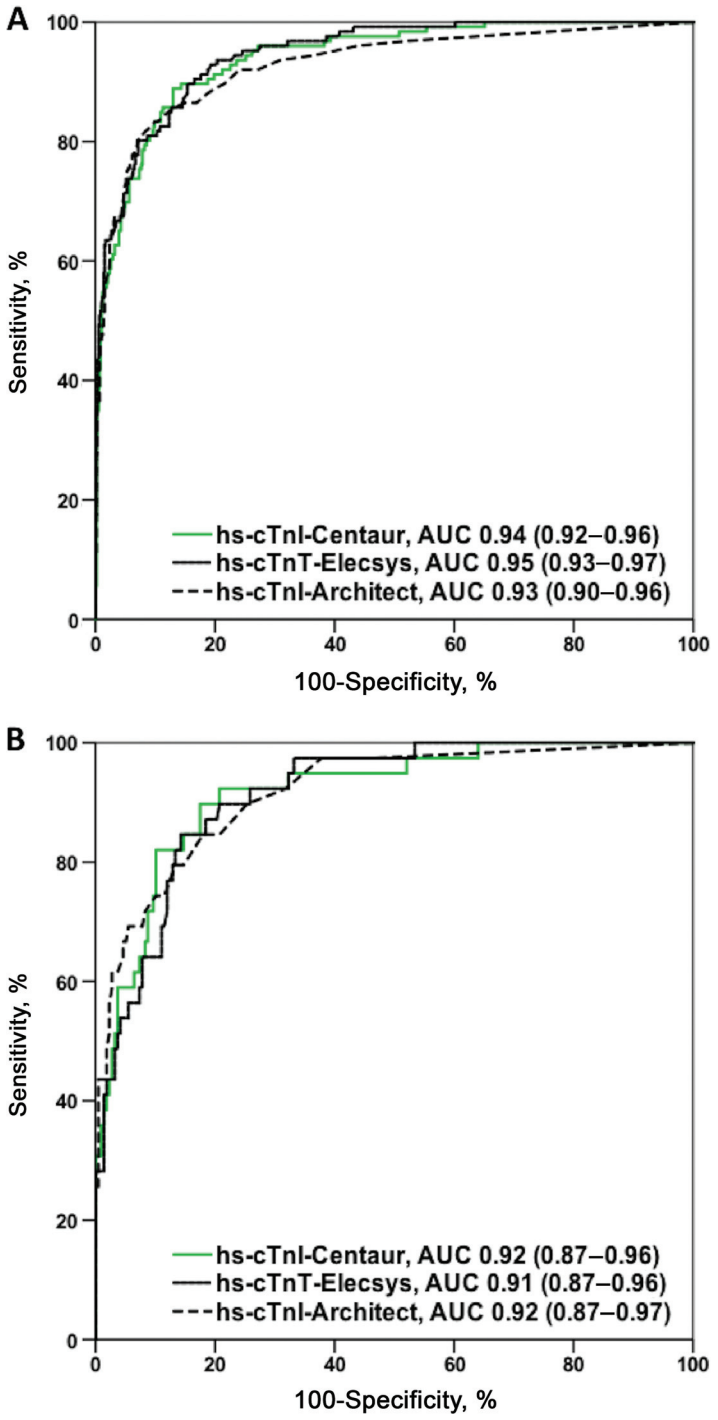

Fig. 2. Diagnostic accuracy of hs-cTn assays at presentation for the diagnosis of AMI.

(A), ROC curves describing the diagnostic performance of the 3 hs-cTn assays at presentation for the diagnosis of AMI. (B), ROC curves describing the diagnostic performance of the 3 hs-cTn assays at presentation for the diagnosis of AMI in patients presenting to the ED within 3 h after chest pain onset.

Table 2. Performance of the hs-cTnI-Centaur 0/1-h algorithm in the validation cohort (n = 675) according to time since CPO.[a]

Time since CPO until first study blood draw	Direct rule-out, 0 h < 3 ng/L (if CPO >3 h)	Overall rule-out, direct rule-out or 0 h < 6 ng/L AND 1 h-delta < 3 ng/L	Observe zone	Direct rule-in, 0 h ≥ 120 ng/L	Overall rule-in, 0 h ≥ 120 ng/L OR 1 h-delta ≥ 12 ng/L
≤1 h (n = 38/675)	NA[b]	19/38 (50%)	11/38 (29%)	3/38 (8%)	8/38 (21%)
		Sens.[c] 100% (61.0–100)	NSTEMI 27%	Spec.[d] 96.9% (84.3–99.4)	Spec. 84.4% (68.2–93.1)
		NPV 100% (83.2–100)		PPV 66.7% (20.8–93.9)	PPV 37.5% (13.7–69.4)
≤2 h (n = 171/675)	NA	79/171 (46%)	56/171 (33%)	17/171 (10%)	36/171 (21%)
		Sens. 100% (89.6–100)	NSTEMI 14%	Spec. 97.8% (93.8–99.3)	Spec. 92.0% (86.3–95.5)
		NPV 100% (95.4–100)		PPV 82.4% (59.0–93.8)	PPV 69.4% (53.1–82.0)
≤3 h (n = 252/675)	NA	117/252 (46%)	83/252 (33%)	28/252 (11%)	52/252 (21%)
		Sens. 100% (92.3–100)	NSTEMI 13%	Spec. 97.1% (93.8–98.7)	Spec. 91.7% (87.2–94.8)
		NPV 100% (96.8–100)		PPV 78.6% (60.5–89.8)	PPV 67.3% (53.8–78.5)
≤4 h (n = 325/675)	16/325 (5%)	151/325 (47%)	112/352 (34%)	33/325 (10%)	62/325 (19%)
	Sens. 100% (93.5–100)	Sens. 100% (93.5–100)	NSTEMI 11%	Spec. 97.4% (94.7–98.7)	Spec. 93.0% (89.3–95.4)
	NPV 100% (80.6–100)	NPV 100% (97.5–100)		PPV 78.8% (62.2–89.3)	PPV 69.4% (57.0–79.4)
≤5 h (n = 380/675)	26/380 (7%)	176/380 (46%)	134/380 (35%)	40/380 (11%)	70/380 (19%)
	Sens. 100% (94.2–100)	Sens. 100% (94.2–100)	NSTEMI 10%	Spec. 97.2% (94.7–98.5)	Spec. 93.4% (90.1–95.6)
	NPV 100% (87.1–100)	NPV 100% (97.9–100)		PPV 77.5% (62.5–87.7)	PPV 70.0% (58.5–79.5)
≤6 h (n = 427/675)	37/427 (9%)	196/427 (46%)	151/427 (35%)	44/427 (10%)	80/427 (19%)
	Sens. 100% (94.8–100)	Sens. 98.6% (92.3–99.7)	NSTEMI 9%	Spec. 97.2% (94.9–98.5)	Spec. 93.0% (89.9–95.2)
	NPV 100% (90.6–100)	NPV 99.5% (97.2–99.9)		PPV 77.3% (63.0–87.2)	PPV 68.8% (57.9–77.8)

[a] Chest pain onset.
[b] Not available.
[c] Sensitivity.
[d] Specificity.

Table 3. Performance of the hs-cTnI-Centaur 0/2-h algorithm in the validation cohort (n = 361) according to time since CPO.[a]

Time since CPO until first study blood draw	Direct rule-out, 0 h < 3 ng/L (if CPO > 3 h)	Overall rule-out, direct rule-out or 0 h < 8 ng/L AND 1 h-delta < 7 ng/L	Observe zone	Direct rule-in, 0 h ≥ 120 ng/L	Overall rule-in, 0 h ≥ 120 ng/L OR 1 h-delta ≥ 20 ng/L
≤1 h (n = 24/361)	NA[b]	16/24 (67%)	3/24 (12%)	2/24 (8%)	5/24 (21%)
		Sens.[c] 100% (43.9–100)	NSTEMI 0%	Spec.[d] 100% (84.5–100)	Spec. 90.5% (71.1–97.3)
		NPV 100% (80.6–100)		PPV 100% (34.2–100)	PPV 60.0% (23.1–88.2)
≤2 h (n = 87/361)	NA	48/87 (55%)	23/87 (26%)	8/87 (9%)	16/87 (18%)
		Sens. 100% (79.6–100)	NSTEMI 9%	Spec. 100% (94.9–100)	Spec. 95.8% (88.5–98.6)
		NPV 100% (92.6–100)		PPV 100% (67.6–100)	PPV 81.3% (57.0–93.4)
≤3 h (n = 133/361)	NA	75/133 (56%)	36/133 (27%)	13/133 (10%)	22/133 (17%)
		Sens. 100% (72.2–100)	NSTEMI 14%	Spec. 100% (96.6–100)	Spec. 96.4% (91.0–98.6)
		NPV 100% (95.1–100)		PPV 100% (77.2–100)	PPV 81.8% (61.5–92.7)
≤4 h (n = 175/361)	11/175 (6%)	102/175 (58%)	45/175 (26%)	17/175 (10%)	28/175 (16%)
	Sens. 100% (87.9–100)	Sens. 100% (87.9–100)	NSTEMI 13%	Spec. 99.3% (96.2–99.9)	Spec. 95.9% (91.4–98.1)
	NPV 100% (74.1–100)	NPV 100% (96.4–100)		PPV 94.1% (73.0–99.0)	PPV 78.6% (60.5–89.8)
≤5 h (n = 204/361)	18/204 (9%)	115/204 (56%)	58/204 (28%)	20/204 (10%)	31/204 (15%)
	Sens. 100% (89.3–100)	Sens. 100% (89.3–100)	NSTEMI 16%	Spec. 98.3% (95.0–99.4)	Spec. 95.3% (91.1–97.6)
	NPV 100% (82.4–100)	NPV 100% (96.8–100)		PPV 85.0% (64.0–94.8)	PPV 74.2% (56.8–86.3)
≤6 h (n = 229/361)	23/229 (10%)	130/229 (57%)	64/229 (28%)	23/229 (10%)	35/229 (15%)
	Sens. 100% (89.9–100)	Sens. 100% (89.8–100)	NSTEMI 14%	Spec. 97.9% (94.8–99.2)	Spec. 94.9% (90.8–97.2)
	NPV 100% (85.7–100)	NPV 100% (97.1–100)		PPV 82.6% (62.9–93.0)	PPV 71.4% (54.9–83.7)

[a] Chest pain onset.
[b] Not available.
[c] Sensitivity.
[d] Specificity.

Table 4. Diagnostic performance of uniform and sex-specific cutoffs.

	Final adjudication including hs-cTnT-Elecsys				Final adjudication including hs-cTnI-Architect			
	Sensitivity	NPV	Specificity	PPV	Sensitivity	NPV	Specificity	PPV
hs-cTnI-Centaur								
Uniform 99th percentile, 47 ng/L	70.4 (65.2–75.2)	93.4 (92.0–94.6)	93.3 (91.9–94.5)	70.0 (64.8–74.8)	75.5 (70.3–80.0)	94.9 (93.6–95.9)	93.5 (92.1–94.6)	70.3 (65.1–75.1)
Men 99th percentile, 58 ng/L	65.2 (58.9–71.1)	92.1 (90.3–93.6)	94.6 (93.0–95.9)	73.9 (67.4–79.5)	70.8 (64.3–76.5)	93.9 (92.2–95.2)	94.7 (93.2–95.9)	73.9 (67.4–79.5)
Women 99th percentile, 39 ng/L	77.3 (67.5–84.8)	95.4 (93.0–97.0)	92.0 (89.1–94.2)	65.4 (55.8–73.8)	80.2 (70.6–87.3)	96.1 (93.8–97.5)	92.3 (89.4–94.4)	66.3 (56.8–74.7)
hs-cTnI-Architect								
Uniform 99th percentile, 26.2 ng/L	77.7 (73.3–81.6)	93.9 (92.6–95.1)	92.5 (91.1–93.8)	68.4 (63.3–73.2)	78.9 (73.9–83.1)	95.5 (94.3–96.5)	92.9 (91.4–94.1)	69.3 (64.2–74.0)
Men 99th percentile, 34.2 ng/L	67.0 (60.6–72.7)	92.4 (90.6–93.9)	94.3 (92.7–95.6)	73.3 (67.0–78.9)	73.1 (66.8–78.6)	94.3 (92.7–95.6)	94.5 (92.9–95.8)	73.8 (67.5–79.3)
Women 99th percentile, 15.6 ng/L	84.1 (75.0–90.3)	96.6 (94.3–97.9)	87.6 (84.2–90.3)	56.9 (48.3–65.1)	88.4 (79.9–93.6)	97.5 (95.5–98.7)	88.1 (84.7–90.7)	58.5 (49.9–66.6)
hs-cTnT-Elecsys								
Uniform 99th percentile, 14 ng/L	94.3 (91.2–96.4)	98.4 (97.5–99.0)	78.1 (75.9–80.2)	48.9 (44.9–52.8)	93.3 (89.9–95.6)	98.2 (97.3–98.9)	76.9 (74.7–79.0)	45.3 (41.40–49.2)
Men 99th percentile, 15.5 ng/L	91.3 (87.0–94.3)	97.5 (96.2–98.4)	80.6 (78.0–83.0)	52.4 (47.5–57.2)	89.6 (84.8–93.0)	97.3 (95.9–98.2)	79.0 (76.4–81.4)	47.4 (42.5–52.3)
Women 99th percentile, 9 ng/L	98.9 (93.8–99.8)	99.6 (97.9–99.9)	58.9 (54.3–63.3)	32.0 (26.7–37.7)	97.7 (91.9–99.4)	99.2 (97.3–99.8)	58.4 (53.8–62.9)	30.9 (25.7–36.6)

adjudication including hs-cTnI (Architect; see Fig. 6 in the online Data Supplement).

DIRECT COMPARISON OF THE hs-cTnI-CENTAUR 0/1-h ALGORITHM WITH THE ESC 0/1-h ALGORITHMS USING hs-cTnT-ELECSYS AND hs-cTnI-ARCHITECT

Overall, the diagnostic performance of the hs-cTnI-Centaur 0/1-h algorithm was similar to that of the hs-cTnT-Elecsys 0/1-h algorithm and the hs-cTnI-Architect 0/1-h algorithm (see Figs. 7 and 8 in the online Data Supplement).

hs-cTnI-CENTAUR 0/2-h ALGORITHM

Optimal thresholds for rule-out and rule-in are shown in Fig. 3C.

VALIDATION OF THE hs-cTnI-CENTAUR 0/2-h ALGORITHM

Applying the derived optimal thresholds to the internal validation cohort, 200 of 361 patients (55%) could be classified as rule-out with a corresponding NPV of 100% and a sensitivity of 100% (95% CI, 94.1–100; see Fig. 3D here and Fig. 9 in the online Data Supplement).

PROGNOSTIC PERFORMANCE OF THE hs-cTnI-CENTAUR 0/1-h ALGORITHM

Median follow-up time was 772 days (IQR, 734–915) with 13 deaths occurring within 30 days and 99 deaths within 2 years. Cumulative 30-day survival rates were 100%, 98.6%, and 97.5% (log-rank, $P = 0.002$) in the rule-out, observe, and rule-in groups, respectively. At 2 years, cumulative survival rates were 98.4%, 89.2%, and 85.1%, respectively (log-rank, $P < 0.001$; Fig. 4). Similar findings emerged regarding MACE-free survival including the index event (see Fig. 10 in the online Data Supplement).

BIOLOGICAL EQUIVALENT CONCENTRATIONS

Biological equivalent concentrations for hs-cTnI-Centaur of hs-cTnI-Architect and hs-cTnT-Elecsys are shown in Fig. 11 of the online Data Supplement.

Discussion

This slarge multicenter study was performed to validate the diagnostic performance and clinical utility of the novel hs-cTnI-Centaur assay for the early diagnosis of AMI. We report 9 major findings.

First, the diagnostic accuracy of hs-cTnI-Centaur was high for concentrations obtained at ED presentation and at 1-h and 2-h changes and their combinations with an AUC ranging from 0.94 to 0.97. Second, the diagnostic accuracy of hs-cTnI-Centaur was comparable with the 2 hs-cTn assays already in clinical use: hs-cTnT-Elecsys and hs-cTnI-Architect. This finding was consistent in the overall population and in early presenters. Third, as compared with the uniform cutoffs, sex-specific cutoffs provided slightly higher sensitivity and NPV but lower spec-

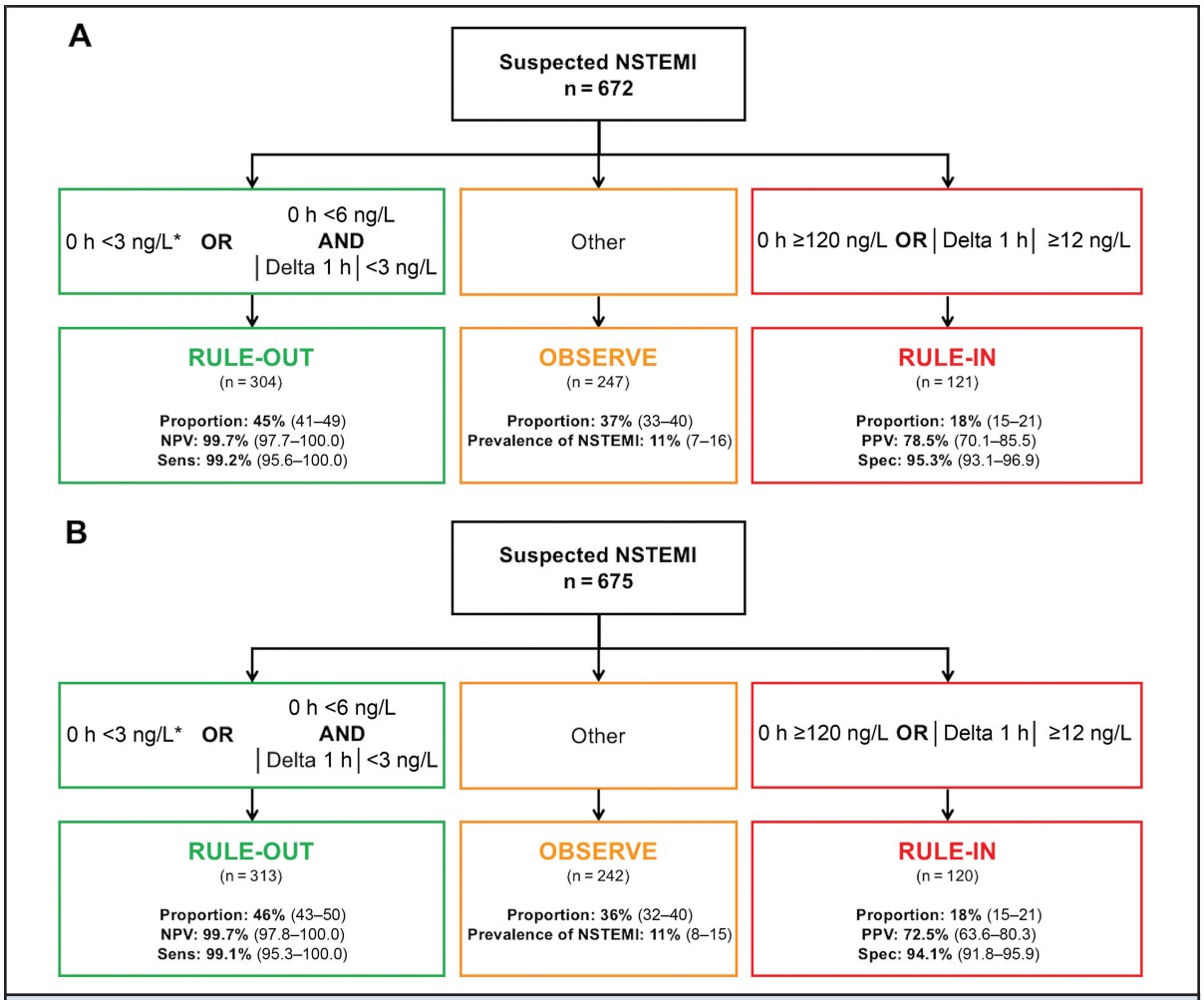

Fig. 3. Performance of the hs-cTnI-Centaur 0/1-h and 0/2-h algorithms in the derivation and validation cohort.

Performance of the hs-cTnI-Centaur 0/1-h algorithm in the derivation cohort (A) and validation cohort (B). |Delta 1h| denotes absolute (unsigned) change of hs-cTnI within 1 h. Performance of the hs-cTnI-Centaur 0/2-h algorithm in the derivation cohort (C) and validation cohort (D). |Delta 2h| denotes absolute (unsigned) change of hs-cTnI within 2 h. NSTEMI, non-ST-elevation myocardial infarction; Sens., sensitivity; Spec., specificity. *If chest pain onset >3h before presentation to the ED.

Continued on page 1357

ificity and PPV in women, and slightly lower sensitivity and NPV but higher specificity and PPV in men. Whereas the use of the sex-specific 99th percentile in women seems reasonable for classification of women at low risk, the use in men seems to be associated with potential harm. Fourth, the application of the derived 0/1-h algorithm for hs-cTnI-Centaur, defined by concentrations at presentation and its absolute change within 1 h, in the internal validation cohort resulted in high safety in the rule-out zone with an NPV of 99.7% and a sensitivity of 99.1%, as well as a high PPV in the rule-in zone for AMI. Only 2 patients were missed, both with decreasing hs-cTn concentrations possibly because of late presentation to the ED. Fifth, overall, the performance of the 0/1-h algorithm for hs-cTnI-Centaur was comparable with that of the established 0/1-h algorithms for hs-cTnT-Elecsys and hs-cTnI-Architect, and also similar to their performance in previous studies *(3, 7, 21)*. In contrast to the established 0/1-h algorithms, the novel hs-cTnI-Centaur 0/1-h algorithm uses a slightly higher cutoff (3 ng/L) for direct rule-out instead of its LoD (2 ng/L). This resulted in a greater proportion of patients ruled out than using the LoD (16% vs 7%; $P < 0.001$). As with most other early rule-out algorithms, the cutoffs for rule-out of AMI with the 0/1-h and 0/2-h algorithms for hs-cTnI-Centaur are low and, therefore, in a range for

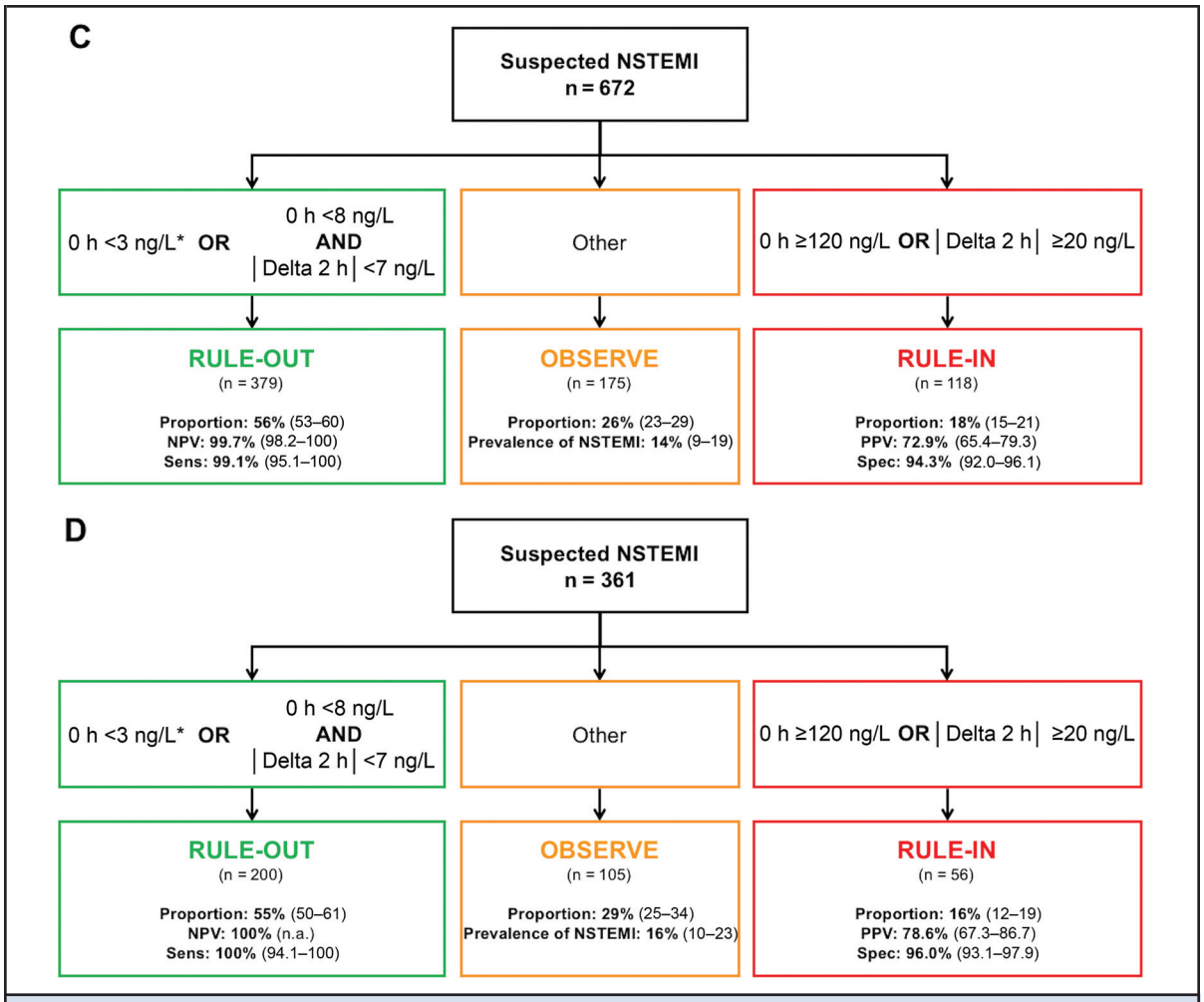

Fig. 3. Continued from page 1356.

which the assay has suboptimal precision. This aspect highlights the need for further independent validation studies. As it is unknown to what extent the analytical qualification as an hs-cTnI assay correlates with the diagnostic accuracy for AMI as quantified by the AUC and the clinical utility as quantified by the performance of the 0/1-h and 0/2-h algorithms, it was mandatory to prospectively evaluate them in this large diagnostic study. Sixth, the application of the derived 0/2-h algorithm for the hs-cTnI-Centaur, defined by concentrations at presentation and its absolute change within 2 h, in the internal validation cohort resulted also in a high NPV and sensitivity of 100%, as well as a high PPV for AMI. Seventh, the overall efficacy of the novel hs-cTnI-Centaur 0/1-h and 0/2-h algorithms was high by assigning about 70% of patients to either rule-out or rule-in within 1 h or 2 h (rule-out efficacy was even higher for the 0/2-h algorithm), with only about 30% of patients remaining in the observe zone. Of note, more than one-fourth (28%) of all patients were either directly ruled out or ruled in for AMI at presentation based on a single hs-cTnI-Centaur concentration without the need for serial hs-cTnI sampling. Eighth, these findings were internally validated when using as an additional reference standard for the second adjudication including serial hs-cTnI concentrations. By the use of a reference standard including hs-cTnI in addition to a reference standard including hs-cTnT, this large diagnostic study of patients presenting with suspected AMI overcame the small but inherent verification bias of previous studies that used only 1 (hs)-cTn assay as part of the reference standard *(7, 11, 19, 23, 30)*. Using the second adjudication resulted in final diagnoses that slightly differed from those using the primary adjudication. This can be explained by the differences among both assays, e.g., by the fact that 99th percentiles are not biologically equivalent to each

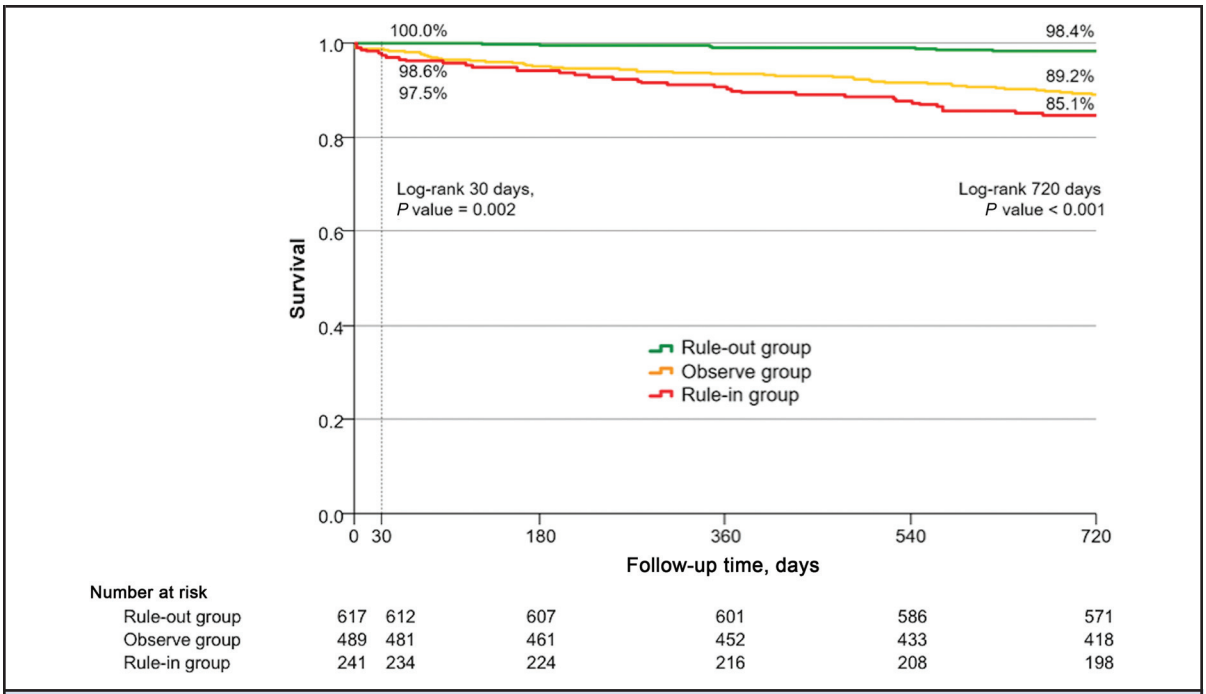

Fig. 4. Short- and long-term survival of patients classified according to the hs-cTnI-Centaur 0/1-h algorithm.
Kaplan–Meier curves depicting overall survival within 30 days and 720 days according to classification of the hs-cTnI-Centaur 0/1-h algorithm.

other. This methodological detail further increases the generalizability of our findings.

Ninth, survival in patients assigned to the rule-out zone by the 0/1-h algorithm was 100% after 30 days and 98.4% after 2 years, further underscoring the safety of early discharge from the ED for most patients classified as rule-out, with further outpatient management as clinically appropriate. Similarly, MACE-free survival within 30 days in patients triaged toward rule-out was high at 99.4%. Of note, the rather high rate of all-cause mortality during follow-up and MACE within 30 days of the observe patients can be, at least in part, explained by the high incidence of chronic diseases, such as chronic heart failure, that are directly associated with high rates of both overall mortality and MACE.

The findings of the present study have enormous clinical implications, as they will allow a substantial number of additional institutions to clinically introduce hs-cTn testing into their treatment of patients with suspected AMI and, thereby, to adopt current clinical practice guideline recommendations without the logistic challenges and costs of introducing an additional analyzer exclusively for the measurement of hs-cTn *(2–4, 10)*. These findings also extend and corroborate previous work with the 2 other hs-cTn assays *(3, 7, 19, 30)*. Accordingly, the same concept and caveats apply to the most appropriate clinical use of any of the 3 hs-cTn assays and their respective 0/1-h and 0/2-h algorithms in the early diagnosis of AMI *(3, 7, 11, 15, 19, 30)*. First, these algorithms should be applied only after ST-segment elevation myocardial infarction (STEMI) has been ruled out by the ECG performed at presentation. Second, although the hs-cTnI-Centaur 0/1-h and 0/2-h algorithm had a high NPV and sensitivity for AMI, they should always be used in conjunction with all other clinical information, including a detailed assessment of chest pain characteristics, physical examination, and the ECG. Additional measurements of hs-cTnI at, e.g., 3 h, are advised whenever the patient remains symptomatic or clinical judgment still points to AMI. These will help to detect the rare but existing phenomenon of delayed release of hs-cTn into the circulation, particularly in early presenters *(3)*. It will also help detect rare but possible errors in the handling of the clinical blood samples, e.g., blood sample of a patient without AMI (and normal hs-cTnI concentrations) erroneously attributed to a patient with AMI. Third, not all patients triaged toward rule-out of AMI are appropriate candidates for early discharge from the ED. Fourth, patients triaged toward rule-in generally are candidates for early coronary angiography. About 75% of patients triaged toward rule-in will be found to have AMI. Most of the remaining patients in the rule-in zone will still benefit from coronary angiography for diagnostic and possible therapeutic purposes, as they may be

found to have takotsubo cardiomyopathy, myocarditis, or unstable angina *(3)*.

Some limitations merit consideration when interpreting these findings. First, this study was conducted in ED patients with symptoms suggestive of AMI. Further studies are required to quantify the utility of rule-out and rule-in strategies for patients with either a higher pretest probability (e.g., in a coronary care unit setting) or for patients with a lower pretest probability (e.g., in a general practitioner setting) of AMI, as well as in the inherently challenging group of critically ill patients. Second, the data presented were obtained from a prospective diagnostic study. Studies applying the diagnostic algorithms prospectively for clinical decision-making are warranted. Third, not all patients with acute chest pain had a second set of laboratory measurements at 1 h and later. The most common reasons for missing blood samples were logistic issues in the ED that precluded blood draw around the 1-h window. This limitation is inherent to studies enrolling consecutive patients and is unlikely to have affected the main findings of the present study. Fourth, although we used the most stringent methodology to adjudicate the presence or absence of AMI, including central adjudication by experienced cardiologists, we still may have misclassified a small number of patients *(4)*. Fifth, our findings are specific to the hs-cTnI-Centaur assay. The derived 0/1-h and 0/2-h algorithms cannot be generalized to other hs-cTnI assays. Sixth, we cannot generalize our findings to patients with terminal kidney failure requiring dialysis because they were excluded from this study. Finally, we acknowledge that using hs-cTnT, an assay that is different from hs-cTnI assays, as the gold standard assay for the primary validation may not be the ideal way to validate an hs-cTnI assay. However, we addressed this potential limitation by use of a secondary adjudication including serial hs-cTnI concentrations.

In conclusion, the diagnostic accuracy of the novel hs-cTnI-Centaur assay for AMI is high and comparable with both well-established hs-cTn assays: hs-cTnT-Elecsys and hs-cTnI-Architect. Simple algorithms incorporating hs-cTnI-Centaur concentrations at presentation and absolute changes within the first 1 h or 2 h allow triage toward safe rule-out and accurate rule-in of AMI within 1 h or 2 h for most patients presenting with chest pain to the ED.

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